CONTACT TIME AND ITS EFFECT ON CROSS-CONTAMINATION OF *ENTEROBACTER AEROGENES* FROM SURFACES TO FOODS

by

ROBYN C. MIRANDA

A Thesis submitted to the

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Master of Science

Graduate Program in Food Science

Written under the direction of

Professor Donald W. Schaffner

And approved by

_____

_____

_____

New Brunswick, New Jersey

[May, 2016]

ABSTRACT OF THE THESIS

Contact Time and its Effect on Cross-contamination of *Enterobacter aerogenes* from Surfaces to Foods

By ROBYN C. MIRANDA

Thesis Director:
Dr. Donald W. Schaffner

Bacterial cross-contamination from surfaces to food can be a common factor contributing to foodborne disease outbreaks, while the popular culture notion of the "five second rule" states food dropped on the floor for less than five seconds is “safe”, because bacteria need time to transfer. The rule has been explored only to a limited degree in the published literature and popular culture. The cross-contamination rate of *Enterobacter aerogenes* was evaluated on common surfaces using scenarios that differ by surface type, food type, contact time (0, 5, 30 and 300 s), and inoculum matrix (TSB or peptone buffer). The surfaces used were stainless steel, ceramic tile, wood and carpet. The food types were watermelon, bread, bread with butter and gummy candy. Surfaces were spot inoculated with 1 ml of inoculum and allowed to dry for 5 h, yielding an approximate concentration of 10^7 CFU/surface. Foods were dropped on the respective surfaces from a height of 12.5 cm and left to rest for the appropriate time. Post transfer surfaces and food were placed in sterile filter bags and homogenized or massaged, diluted and plated on tryptic soy agar. The transfer rate was quantified by determining the log % transfer from the surface to the food. Contact time, food and surface type all had a highly significant effect ($P < 0.000001$) on log % transfer of *Enterobacter aerogenes* from surface to food. The inoculum matrix (TSB or peptone buffer)

also had a significant effect on transfer (P = 0.012944), and most of the interaction terms had a significant effect on transfer. More bacteria transferred to watermelon (~ 0.2-97%) relative to other food types studied, while fewer bacteria transferred to gummy candy (~0.1-62%). Transfer of bacteria to bread (~0.02-94%) and bread with butter (~0.02-82%) were similar, and transfer rates under a given set of condition were more variable compared to watermelon and gummy candy.

Dedication

This thesis is dedicated to my parents, Ron and Sondra Miranda, for their endless love, support and encouragement and whose good examples have taught me to work hard for the things that I aspire to achieve. I am truly thankful for having you in my life. This work is also dedicated to my brother, Joe, who has been a constant source of support and encouragement during the challenges of graduate school.

Acknowledgments

I would like to sincerely thank my advisor, Dr. Schaffner, for his guidance and support throughout my undergraduate and graduate studies, and especially for his confidence in me. He has helped me realize my potential both in and outside of academia, and he always provided honest criticism.

I would like to thank my committee members, Dr. Chikindas and Dr. Tepper, for their critique of my thesis and their time.

I would like to thank my lab mates Dane, Ann, Jenny, and Jiin who have always given me advice and who I have come to consider close friends.

I would like to thank the undergraduates, who helped me with my lab work. Gabriella, Meghan, Rachel, Nimit, Erika thank you for your time and patience. I can only hope I taught you as much as you have taught me.

Finally I would like to thank the staff at food science, Debbie, Irene, Laura, Rosanne, Madelaine, Yakov, Bill and Dave, for all of their help.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

CDC- Centers for Disease Control and Prevention

CFU- colony forming unit

FDA- U.S. Food and Drug Administration

PEP- Peptone water

TSA- Tryptic Soy Agar

TSA-na- Typtic Soy Agar with nalidixic acid

TSB- Tryptic Soy Broth

TSB-na- Typtic Soy Broth with nalidixic acid

USDA- United States Department of Agriculture

Chapter I – Literature Review

I.1 Prior research on the five-second rule

The popular culture notion of the "five second rule" states food dropped on the floor for less than five seconds is “safe”, because bacteria need time to transfer. The rule has been explored to a limited degree in the published literature and popular culture. Previous studies on the “five second rule” use different surfaces, foods, organisms, contact times and number of replicates, making comparisons and conclusions difficult. The first known research recorded on this topic was performed at the University of Illinois, but was never published in the peer-reviewed literature (*4*). These researchers used tile inoculated with *Escherichia coli* and studied transfer to cookies and gummy bears and found that bacterial transfer was observed in less than 5 seconds (*4*). The popular television show MythBusters aired an episode on the “five second rule” in 2005, and found no conclusive difference when comparing contact times of 2 and 6 seconds (*5*). In the only peer-reviewed research on the topic, researchers from Clemson University concluded that longer times (5, 30 and 60 sec) did increase the transfer of *Salmonella* Typhimurium from wood, tile or carpet to bologna or bread but only ≥ 8 h after the surface was inoculated (*29*). Researchers at Aston University in the United Kingdom, published a press release in 2014 showing that contact time significantly affected transfer of both *E. coli* and *S. aureus* contaminated surface (carpet, laminate and tile) to food (toast, pasta, biscuit and a sticky sweet) (*42*). Discovery Science Channel's "The Quick and the Curious" television show aired a short segment offering up cookies to strangers in a park – after dropping them onto the ground. The shows narrator

stated "Moist foods left longer than 30 seconds collect 10 times the bacteria than those snapped up after only three" but offered no data in support of this statement (*76*).

I.2 Outbreaks linked to cross-contamination

The Centers for Disease Control and Prevention (CDC) estimates that each year there are more than 9 million episodes of foodborne illness, over 55 thousand hospitalizations and at least 1,351 deaths that can be attributed to foods consumed in the US (*90*). Cross-contamination is an important factor contributing to foodborne illness, along with failure to cool and reheat foods properly (*13*, *38*). Cross-contamination is the process where bacteria are unintentionally transferred from one person, object or place to another (*77*).

The CDC regularly publishes reports that summarize data on surveillance for foodborne disease outbreaks in the US (*19-22*, *46*). Those reports list more than 30 contributing factors linked to foodborne disease outbreaks in the year or years summarized in the reporting period. Factors are grouped into 3 categories related to contamination, proliferation or survival of foodborne pathogens. Several contamination factors are related to barehanded, glove-handed or other contamination by food handler or others suspected to be infectious. One factor is specifically related to cross-contamination of ingredients (where cross-contamination does not mean by ill food workers), and when those data are summarized from 1998 to present, about 12% of all outbreaks reported to the CDC are linked in some way to this type of cross-contamination, and this is the 6th (out of 32) most common contributing factor (*19-22*, *46*).

I.3 Cross-contamination research

Cross-contamination research has been conducted using various methods. Some vary in their direction of transfer (e.g. surface to food, food to surface, hands to food, etc.). The transfer of

organisms from one surface to another depends on factors such as the contact pressure, initial inoculum size, contact time, surface type, surface moisture and friction (*10*, *29*, *51*, *58*, *68*, *70*, *71*, *89*).

I.4 Microorganism characteristics related to cross-contamination

I.4.a Bacteria attachment properties

Knowledge of bacterial attachment is essential in understanding cross-contamination. Attachment is a complex process and surface structures, surface charge, moisture and abrasiveness of the surface all play a role (*32*, *40*, *65*, *69*, *81*, *107*). The attachment of bacterial cells is in part, dependent on the presence of flagella and fimbrae, production of extracellular polymeric substances and the hydrophobicity of the surface as well (*31*, *32*). Fimbriae are proteinaceous surface appendages (*56*) found in gram-positive and gram-negative bacteria that contribute to cell surface hydrophobicity. Fimbriae attach to surfaces by overcoming electrostatic repulsion between the cell and substratum (*26*, *32*). Flagella provide motility for the bacteria to find attachment areas, although they apparently do not act specifically as an adhesive (*32*). Adhesive structures are usually not expressed at the same time as flagella so that movement and attachment can occur separately (*48*). Hydrophobic interactions typically increase with an increasing nonpolar nature of the surfaces involved (*32*). Strong hydrophobic properties can increase bacterial adherence to surfaces, but adherence is not solely based on hydrophobicity (*37*, *107*).

I.4.b Biofilm Formation

Biofilms are collections of microorganisms where cells are adhered to one another and to a surface. Cells in a biofilm may be contained in a matrix of extracellular polymeric substance produced by the cells during biofilm formation. Biofilms can form on living and non-living surfaces and its formation can be relevant to the study of microbes in natural, food

processing, engineering and biomedical sciences (*25*). Biofilms begin to form when microorganisms attach to the surface by physical forces including van der Waals forces, steric interactions and electrostatic interactions (*43*, *50*, *52*). As the bacteria begin to grow and multiply in association with the surface, they secrete a polysaccharide that may help anchor them. Cells in biofilms may act as a single living organisms, moving across a surface or detaching and spreading to colonize additional surfaces. Biofilms provide the bacteria in them with protection from adverse environmental conditions, antibiotics (*45*), and disinfectants (*79*).

I.4.c *Enterobacter aerogenes*

Enterobacter aerogenes is a nonpathogenic bacterium with attachment characteristics similar to that of *Salmonella spp*. (*24*, *119*). The food grade strain used in our research has been developed to remove free sugars in dried egg products to prevent Maillard browning during storage (B199A Vivolac Cultures, Indianapolis, Ind.) (*119*). This *E. aerogenes* strain is used as a surrogate for known foodborne pathogens.

I.5 Surfaces

I.5.a Stainless Steel

Stainless steel is commonly used in environments where there is a risk for cross-contamination to occur (*116*). These environments include domestic kitchens, hospitals, food manufacturing plants and many more (*116*). Stainless steel has been reported to have higher bacterial transfer rates, when compared to other surfaces (*57*, *86*, *115*). Stainless steel has often been considered the optimal material choice for kitchen sinks and food preparation surfaces due to its resistance to corrosion, mechanical strength, ease of cleaning and its resistance to chemical degradation (*49*, *116*). Because of these attributes, stainless steel is more likely to maintain its hygienic properties after many uses (*49*, *94*).

I.5.b Tile

Tile of various types is commonly found in homes. Unglazed tiles are typically thicker and denser then glazed tiles. Unglazed tiles provide a more slip resistant surface due to their unfinished exteriors. Glazed tiles are more resistant to staining than unglazed tiles because of the protection by the non-porous glaze layer. Ceramic tiles have been developed that have performance features that make them appealing for industrial use. Some of these characteristics include self-cleaning, hygienic, bactericidal and anti-grease properties (*9*). The coatings on ceramic tile tend to be hydrophilic. Water spreads well on such ceramic tiles allowing for "self-cleaning", such that humidity in the air forms a film on the tile which prevents dirt from settling. In applications where microbial growth control is needed, specific bactericidal coatings can be used (*9*).

I.5.c Wood

Wood surfaces are commonly found in households, either as flooring or as cutting board surfaces. The sanitary properties of wood cutting boards have been compared to plastic cutting boards (*2*, *113*), and have come to contradictory conclusions in part due to differences in the methods. Most methods are representative of cutting boards in a home setting, rather then on a commercial scale. Gilbert and Watson found that wood was harder to clean versus plastic cutting boards when ground beef was pressed on the surface (*44*). Wooden surfaces have a high porosity and absorbency that allow for retention of microbial contaminants (*1*). A survey by the U.S. Food and Drug Administration (FDA) found that 25% of consumers did not clean a cutting board after using it to cut raw meat or chicken (*55*). The United States Department of Agriculture (USDA) recommends one cutting board for produce and bread and a separate cutting board for raw meat, poultry and seafood (*103*).

I.5.d Carpet

Carpet serves multiple uses in households including as insulation, to dampen noise and to minimize injury due to falls. Carpet is most often found in bedrooms and living rooms, not kitchens; but it may still be present in locations where food is consumed. The microbiology of carpets and carpet cleaning has been a concern, specifically in hospital settings, as it can act as a reservoir for microorganisms (*3*, *6*, *47*, *60*, *62*, *67*, *87*, *93*). Carpet is not easily cleaned when contaminated with bacteria (*83*). Carpets do accumulate more bacteria than uncarpeted flooring, and bacteria in carpets can aerosolize (*3*, *110*), although, no epidemiological evidence indicates that hospitals with carpet have an increased infection rate (*3*). A norovirus outbreak in a UK hospital showed that virus particles survived in a carpet for 12 days, were not removed by standard vacuum cleaning, and infected two workers who replaced the contaminated carpet (*23*). Microorganisms on carpet can be controlled by specific chemical treatments of the fibers or the materials used in constructing the carpet (*117*) Microbes present in carpet not only pose a cross-contamination risk to food, but can also lead to foul odors and eventual deterioration of the carpet.

I.6 Food

I.6.a Watermelon

Foodborne disease outbreaks have become more frequently associated with produce (*96*). Melons are eaten throughout the world and they are commonly consumed raw (*41*). Melons may be contaminated during production because they are in direct contact with the soil, a potential source of contamination (*41*, *84*). Contamination may also occur during harvest, packing, shipping, or during preparation for consumption (*28*). Melons were identified in 85 outbreaks from 1950 to 2011 with majority of outbreaks occurring in North America. Following cantaloupe (24.7%), watermelon (16.5%) was the next most common melon

implicated as the food vehicle in outbreaks (*41*). An outbreak of *Salmonella* Oranienburg in 1979 occurred with 18 cases traced to precut watermelons (*16*). The use of damaged precut watermelons, covered with plastic film and stored without refrigeration until sold, were factors contributing to the outbreak (*11*, *16*, *17*). A multistate outbreak occurred in 2012 involving *Salmonella* Typhimurium and *Salmonella* Newport occurring in whole cantaloupe in which a recall was extended to watermelons from the same farm (*18*). Whole melons are typically stored at room temperature at supermarkets and in the home, but cut produce should be refrigerated due to the risk of bacterial growth and survival (*8*, *12*, *35*, *102*, *105*). Cut or bruised produce surfaces are more susceptible to bacterial adhesion over undamaged produce surfaces, which can increase the risk of outbreaks from fresh cut produce (*95*).

I.6.b Bread

Bread is an essential dietary staple across the world, prepared from the dough of cereal flour. Bread is either unleavened or fermented using yeasts or a mixture of lactic acid bacteria and baked. Bread has two macroscopic phases: a solid (cell wall material) and a fluid (air) (*14*, *91*). The nature of how the two phases connect determines the structural and mechanical properties of the bread (*99*, *112*). A significant microbiological property of bread is its high water activity (0.94-0.97) and moisture content (40%) (*27*, *63*, *73*). Bread is baked between 190 and 260°C, yet the center does not exceed 97°C, making the bread susceptible to spoilage by mold (*63*) or spore-forming *Bacillus* spp. that can survive the baking process (*36*). Although bread is typically distributed at room temperature and is susceptible to microbial growth, it generally does not cause a public health concern. Baked products are not commonly associated as being vehicles for foodborne illness. However, a multistate outbreak of *Salmonella* Thompson in 2000 was linked to commercially distributed bread. The outbreak was traced back to an ill bakery worker who handled bread in a bakery supplying

bread and buns to restaurants (*54*, *98*). An outbreak of 43 Hepatitis A cases was linked to an ill UK food worker who wrapped bread and sandwiches for sale (*98*, *111*).

I.6.c Butter

The composition of butter consists of milk fat, non-fat milk solids, salt (optional) and water. Colors, neutralizers and lactic starter cultures may be used during the manufacturing process as well. Butter is a water-in-oil emulsion where the water is present in small droplets. The microbiological stability of butter is dependent on if the water is dispersed in small or large droplets (*106*). Butter manufacturing is a microbiologically sensitive process and hygienic procedures must be observed. Butter and whipped butter were linked to staphylococcal foodborne disease outbreaks involving over 100 people in the 1970's (*15*, *101*). A 1991 outbreak from blended margarine and butter contaminated with *S. intermedius* affected more than 265 people in the U.S. (*53*, *104*). Butter contaminated with *Listeria monocytogenes* resulted in 4 deaths in the late 1990's in Finland (*66*). Green butter (made with *Citrobacter freundii* contaminated parsley) sickened more than 150 children at a German nursery school in the mid-1990s (*100*), and garlic butter contaminated by *Campylobacter jejuni* involving 30 people at a US restaurant in 1995 (*118*). The water activity of butter is approximately 0.97 and its continuous fat phase make it susceptible to the growth of microorganisms (*39*, *64*).

I.6.d Gummy candy

Conventionally, gummy candies are made from a mixture of sugar, glucose syrup, starch, flavoring, food coloring, citric acid and gelatin. Gelatin is a polymer that is made from natural sources; it is made of proteins (18 amino acids) and has a low melting point, allowing it to be easily formed with heating (*78*). Collagen is processed into gelatin by an acidic or basic pretreatment, resulting in different isotonic points. Gelatin is used as a binder in gummy candy products. Competition between gelatin and glucose syrup may occur and can

result in a loss of gelling or hardness of the product. Gelatin is also suitable for immobilizing Gram-positive and Gram-negative bacteria (*82*). Bacterial adhesion to gelatin is theorized to occur in two stages: the initial, reversible attachment and the stable, irreversible attachment. Rinsing gelatin has shown to remove the bacteria in the initial stages of adhesion, whereas irreversible attachment has been shown to occur within minutes of contact (*82*). The water activity of confectionary jellies ranges from 0.65-0.75 (*85*), and foods with a water activity below 0.85 but greater than 0.60 are susceptible to spoilage by xerophilic molds and osmophilic yeasts (*108*). The water activity of these products is most influential on the type of microorganism that will be associated with spoilage (*61*), and controlling the moisture content helps prevent spoilage of confectionery products susceptible to mold growth. There have been no reported food poisoning cases linked to the consumption of gummy confectionery products to date (*97*).

I.7. Summary

As noted above, the popular culture notion of the "five second rule" states food dropped on the floor for less than five seconds is “safe”, because bacteria need time to transfer. Since the rule has been explored to only a limited degree in the published literature and popular culture, often using inconsistent methods or incomplete experimental designs, the research in this thesis seeks to quantify cross-contamination between a variety of foods and common kitchen surfaces varying time and bacterial matrix. The results described below will advance our understanding of cross-contamination and the factors that influence it.

CONTACT TIME AND ITS EFFECT ON CROSS-CONTAMINATION AND CONTACT TIME OF *ENTEROBACTER AEROGENES* FROM SURFACES TO FOODS

Robyn C. Miranda and Donald W. Schaffner

Rutgers, The State University of New Jersey

65 Dudley Road, New Brunswick, NJ, 08901

Key words: cross-contamination, contact time, surface, 5-second rule

To Be Submitted To: Journal of Food Protection

Chapter II - Contact Time and its Effect on Cross-contamination of *Enterobacter aerogenes* from Surfaces to Foods

II.1 Abstract

Bacterial cross-contamination from surfaces to food can be a common factor contributing to foodborne disease outbreaks, while the popular culture notion of the "five second rule" states food dropped on the floor for less than five seconds is “safe”, because bacteria need time to transfer. The rule has been explored only to a limited degree in the published literature and popular culture. The cross-contamination rate of *Enterobacter aerogenes* was evaluated on common surfaces using scenarios that differ by surface type, food type, contact time (0, 5, 30 and 300 s), and inoculum matrix (TSB or peptone buffer). The surfaces used were stainless steel, ceramic tile, wood and carpet. The food types were watermelon, bread, bread with butter and gummy candy. Surfaces were spot inoculated with 1 ml of inoculum and allowed to dry for 5 h, yielding an approximate concentration of 10^7 CFU/surface. Foods were dropped on the respective surfaces from a height of 12.5 cm and left to rest for the appropriate time. Post transfer surfaces and foods were placed in sterile filter bags and homogenized or massaged, diluted and plated on tryptic soy agar. The transfer rate was quantified by determining the log % transfer from the surface to the food. Contact time, food and surface type all had a highly significant effect ($P < 0.000001$) on log % transfer of *Enterobacter aerogenes* from surface to food. The inoculum matrix (TSB or peptone buffer) also had a significant effect on transfer ($P = 0.012944$), and most of the interaction terms had a significant effect on transfer. More

bacteria transferred to watermelon (~ 0.2-97%) relative to other food types studied, while fewer bacteria transferred to gummy candy (~0.1-62%). Transfer of bacteria to bread (~0.02-94%) and bread with butter (~0.02-82%) were similar, and transfer rates under a given set of condition were more variable compared with watermelon and gummy candy.

II.2 Introduction

The Centers for Disease Control and Prevention (CDC) estimates that each year there are more than 9 million episodes of foodborne illness, over 55 thousand hospitalizations and at least 1,351 deaths that can be attributed to foods consumed in the US (*90*). The CDC regularly publishes reports that summarize data on surveillance for foodborne disease outbreaks in the US (*19-22*, *46*). Those reports list more than 30 contributing factors linked to foodborne disease outbreaks in the year or years summarized in the reporting period. Factors are grouped into 3 categories related to contamination, proliferation or survival of foodborne pathogens. Food handlers or others suspected to be infectious are linked to several contamination factors. One factor is specifically related to cross-contamination from surfaces and not ill individuals. When those surface cross-contamination data are summarized from 1998 to present, about 12% of all outbreaks reported to the CDC are linked in some way to this type of surface cross-contamination. This is the 6th most common contributing factor (out of 32) (*19-22*, *46*).

Household and other surface types have been a focus of numerous cross-contamination studies; surfaces studied include ceramic tile (*29*, *51*, *114*), stainless steel (*51*, *59*, *72*, *75*, *114*), wood (*29*), glass (*51*), plastic (*24*, *51*, *119*) and carpet (*29*, *60*, *83*). Stainless steel has often been considered the optimal material choice for kitchen sinks and commercial food preparation surfaces due to its resistance to corrosion, mechanical strength, ease of cleaning and its resistance to chemical degradation (*49*, *116*), although stainless steel may have higher bacterial transfer rates when compared to other surfaces (*57*, *86*, *115*). Tile is also a common surface found in homes; the variations of tile (unglazed versus glazed) may have an affect on the bacterial transfer rate because of varying topography (*9*).

Wood surfaces are commonly found in households, either as flooring or as cutting board surfaces. The sanitary properties of wood cutting boards have been compared to plastic cutting boards (*2*, *113*), and have come to contradictory conclusions in part due to differences in the methods. The United States Department of Agriculture (USDA) recommends one cutting board for produce and bread and a separate cutting board for raw meat, poultry and seafood (*103*). Carpet is a likely site of contamination in the household and inactivating or removing bacteria using conventional cleaning methods is difficult once the carpet is contaminated (*83*). Microorganisms on carpet can be controlled by specific chemical treatments of the fibers or the materials used in constructing the carpet (*117*).

The popular culture notion of the "five second rule" states food dropped on the floor for less than five seconds is “safe”, because bacteria need time to transfer. The rule has been explored to a limited degree in the published literature and popular culture. Previous studies on the “five second rule” use different surfaces, foods, organisms, contact times and number of replicates, making comparisons and conclusions difficult. The first known research recorded on this topic was performed at the University of Illinois, but was never published in the peer-reviewed literature (*4*). These researchers used tile inoculated with *Escherichia coli* and studied transfer to cookies and gummy bears and found that bacterial transfer was observed in less than 5 seconds (*4*). The popular television show MythBusters aired an episode on the “five second rule” in 2005, and found no conclusive difference when comparing contact times of 2 and 6 seconds (*5*). In the only peer-reviewed research on the topic, researchers from Clemson University concluded that longer times (5, 30 and 60 sec) did increase the transfer of *Salmonella* Typhimurium

from wood, tile or carpet to bologna or bread but only ≥ 8 h after the surface was inoculated (*29*). Researchers at Aston University in the United Kingdom, published a press release in 2014 showing that contact time significantly affected transfer of both *E. coli* and *S. aureus* contaminated surface (carpet, laminate and tile) to food (toast, pasta, biscuit and a sticky sweet) (*42*). Discovery Science Channel's "The Quick and the Curious" television show aired a short segment offering up cookies to strangers in a park – after dropping them onto the ground. The shows narrator stated "Moist foods left longer than 30 seconds collect 10 times the bacteria than those snapped up after only three" but offered no data in support of this statement (*76*).

As noted above, the popular culture notion of the "five second rule" states food dropped on the floor for less than five seconds is “safe”, because bacteria need time to transfer. Since the rule has been explored to only a limited degree in the published literature and popular culture, often using inconsistent methods or incomplete experimental designs, the research in this thesis seeks to quantify cross-contamination between a variety of foods and common kitchen surfaces varying time and bacterial matrix. The results described below will advance our understanding of cross-contamination and the factors that influence it.

II.3 Materials and Methods

II.3.a Bacterial strain and preparation of culture

A nonpathogenic, food-grade microorganism, *Enterobacter aerogenes* B199A, with attachment characteristics similar to *Salmonella,* was used for all experiments (Vivolac Cultures, Indianapolis, Ind.) (*119*). The *E. aerogenes* strain is resistant to nalidixic acid, which allows it to be enumerated in the presence of other microorganisms on the food

samples or surfaces. Control experiments showed that nalidixic acid-resistant *E. aerogenes* cells were not initially present on any of the foods or surfaces.

Cultures were prepared based on prior work in our lab (*24*) and by others (*119*). Frozen stock of *E. aerogenes* in 80% sterile glycerol was streaked onto tryptic soy agar, (Difco, BD, Sparks, MD) with 50 μg/ml nalidixic acid (Sigma Chemical Co., St. Louis, Mo.) (TSA-na). One colony from each plate was transferred to 10 ml of tryptic soy broth (Bacto, BD, Sparks, MD) with 50 μg/ml nalidixic acid (TSB-na) and incubated at 37°C for 24 h. Inoculum matrices were of two types; using cells harvested by centrifugation at 5,000 ×g for 10 min and washed twice in 10 ml of 0.1% peptone (Difco, BD) or using cells taken directly from inoculated, overnight TSB-na culture. A final concentration of 10^7 CFU/ml was verified by enumeration on TSA-na.

II.3.b Preparation of domestic surfaces

Four different surfaces typical of those found in domestic environments were used: stainless steel (.018" thickness, 16 gauge; onlinemetals.com, Seattle, WA), ceramic glazed tile (Brancacci Windrift Beige, Daltile, Dallas, TX), maple laminate wood (Northern Maple, Mohawk, Calhoun, GA) and indoor/outdoor carpet (Morella, Foss Manufacturing, Hampton, NH) were ordered online or purchased from a local home improvement store. Surface materials were cut into coupons (5 x 5 cm). The stainless steel and ceramic tile coupons were disinfected prior to inoculation by soaking in 70% ethanol for 1 h, removed, air-dried and autoclaved. Disinfection of wood and carpet coupons caused structural changes so these were discarded after autoclaving following single use. Control experiments showed that nalidixic acid-resistant *E. aerogenes* cells were not initially present on any surfaces.

II.3.c Food types

Four foods (watermelon, white bread (ShopRite, Wakefern Food Corp., Elizabeth, NJ), unsalted butter (ShopRite, Wakefern Food Corp., Elizabeth, NJ) and gummy candy (Haribo, Strawberries)) were purchased online or from a local supermarket. Whole watermelon was stored at 4°C prior to use. The watermelon (flesh only) and bread (excluding crust) were cut into pieces (approximately 4 by 4 cm). Unsalted butter was brought to ambient temperature (~24°C) prior to spreading onto bread. All foods had equivalent contact areas (~16 cm^2). The pH and water activity of samples were measured using a surface pH probe (Accumet Basic AB15 pH Meter, Fisher Scientific) and water activity meter (Rotronic Instrument Corp., Hauppauge, NY) respectively.

II.3.d Transfer between food and surfaces

Transfer scenarios were evaluated for each contact surface type (4), each food type (4), four contact times and two inoculum matrices, totaling 128 scenarios. Each scenario was replicated 20 times, totaling 2,560 measurements. Each contact surface type was spot inoculated with 1 ml of inoculum using eight to ten drops spread over the 5 x 5 cm surface. The surfaces were placed in a biosafety cabinet (SterilGARD Hood, The Bakery Company, Inc., Sanford, ME) for 5 h, after which the surface was visibly dry. Both the peptone buffer and TSB-na inoculum matrices yielded an approximate concentration of 10^7 CFU/surface after drying. Foods were dropped on the respective surfaces from a height of 12.5 cm and left to rest for four different times (0, 5, 30 and 300 s). Surfaces were placed into a sterile Whirl-Pak filter bag (Nasco, Fort Atkinson, WI), 20 ml of peptone buffer was added, and hand massaged for 2 min. Foods were placed into a sterile filter bag (Fisherbrand, Lab Blender Bags) with 50 ml of peptone buffer and the

samples were homogenized (Stomacher, Cooke Laboratory Products, Alexandria, VA) for 3 min. Surfaces and food samples were serially diluted in 0.1% peptone buffer and surface plated (0.1 ml) onto TSA-na for enumeration of *E. aerogenes.* Plates were incubated at 37°C for 24 h. Colonies were counted and population levels were expressed as CFU per food or surface sample.

II.3.e Data analysis

Percent transfer was calculated as:

[Total CFU food] / [Total CFU food + Total CFU surface] × 100

Percent transfer rates from surface to food were log transformed using Microsoft Excel (Microsoft, Redmond, WA) and Sigma Plot (Systat Software Inc., San Jose, CA), as prior research has shown that untransformed transfer rates are highly skewed, and log transformed transfer rates are approximately normally distributed (*24*, *92*). Multiple linear regression analysis was performed using StatPlus for Microsoft Excel (AnalystSoft, Inc., Walnut, CA). Variables and the interactions between variables were considered significant when $P < 0.05$.

II.4 Results

II.4.a pH and Water Activity (a_W) Measurements

The pH and water activity (a_W) measurements for all food types are shown in Table 1. Watermelon had the highest a_W of the foods studied. Bread and butter had measured a_W values close to watermelon. The a_W of the gummy candy was considerably lower than that of the other foods measured (0.72 vs. ≥ 0.95). Butter had the highest pH (6.25) of any of the foods measured and gummy candy had the lowest (2.80). Although low pH is known to cause stress injury to microorganisms, it is unlikely given the short contact time

in this study that this would have occurred in the gummy candy experiments (*7*). The measured pH values of bread and watermelon were intermediate (5.80 and 5.43, respectively).

II.4.b Statistical analysis of transfer rates

The result of the multiple linear regression analysis is shown in Table 2. For *E. aerogenes,* the contact time, food, surface and the food*time interaction was shown to significantly ($P < 0.000001$) influence log % transfer. The surface*time ($P = .001896$), surface*food ($P = 0.000190$) and surface*matrix ($P = 0.000050$) effect on log % transfer were also significant. The inoculum matrix, i.e. TSB or buffer ($P = 0.012944$) and food*matrix interaction ($P = 0.044589$) were statistically significant, although less so than the other factors. The time*matrix interaction did not have a statistically significant effect on log % transfer ($P = 0.494994$) (Table 2). This means that while both time and inoculum matrix may influence transfer rate, of those interactions are independent such that there is no additional effect from the two factors working together.

Transfer of bacteria from inoculated surfaces to watermelon, bread, bread with butter and gummy candies, is summarized in Tables 3, 4, 5 and 6, respectively. Each table shows six different statistical parameters that were used to characterize the log % transfer rate: mean ($\bar{x}$), median (M), standard deviation (σ), minimum (min), maximum (max) and range. The tables will be referenced as needed to supplement the discussion of the figures below.

II.4.c Bacteria transfer from inoculated surface to food

The transfer of *E. aerogenes* from TSB and buffer inoculated surfaces (tile, stainless steel, wood and carpet) to food (watermelon, bread, bread with butter and gummy candy)

Table 1. pH and Water Activity measurements of four foods to which *Enterobacter aerogenes* are transferred from common household surfaces.

Food type	Water Activity	pH
Bread	0.95	5.80
Butter	0.97	6.25
Gummy	0.72	2.80
Watermelon	0.99	5.43

Table 2. Multiple Linear Regression analysis results for the effects of contact time, inoculum matrix, food type, surface type, and their interactions on the transfer of *Enterobacter aerogenes* from common household surfaces to foods.

	Coefficient	Standard Error	LCL	UCL	t Stat	p-level
Intercept	0.38	0.09	0.20	0.56	4.18	0.000030
Time	0.01	0.00	0.01	0.01	13.40	<0.000001
Matrix	-0.26	0.11	-0.47	-0.06	-2.49	0.012944
Food	0.23	0.04	0.15	0.32	5.36	<0.000001
Surface	-0.25	0.04	-0.33	-0.16	-5.78	<0.000001
Time*Matrix	0.00	0.00	0.00	0.00	-0.68	0.494994
Time*Food	0.00	0.00	0.00	0.00	-7.90	<0.000001
Time*Surface	0.00	0.00	0.00	0.00	-3.11	0.001896
Matrix*Food	-0.08	0.04	-0.17	0.00	-2.01	0.044589
Matrix*Surface	-0.17	0.04	-0.25	-0.09	-4.06	0.000050
Food*Surface	0.07	0.02	0.03	0.11	3.74	0.000190

over time (0, 5, 30 and 300 s) is shown in Figure 1 and 2, respectively. Figure 1 shows the transfer of *E. aerogenes* from inoculated surfaces to food. Figure 2 shows the transfer of *E. aerogenes* from buffer inoculated surfaces to food. Error bars in Figures 1 and 2 indicate the standard deviation of the recorded observations. Since many scenario results were similar, not all observations will be specifically discussed below.

II.4.d Inoculated surface to watermelon

When all TSB inoculated surfaces contacted watermelon, a high degree of transfer of bacteria to watermelon occurred (Figure 1, Table 3). Log % transfer of bacteria from tile to watermelon for cells contained within the TSB inoculum was highest at 5 s with 1.99 log % transfer (97.15%) (Table 3, Figure 1M). Transfer of bacteria from stainless steel was between 1.96 (90.21%) and 1.97 log % transfer (92.73%) (Table 3, Figure 1N). Overall, there was no significant difference in bacterial transfer from any surface to watermelon at different contact times (Table 3, Figure 1 MNOP).

Bacterial transfer from buffer inoculated surfaces to watermelon was more variable then the TSB inoculum matrix (Table 3, Figure 2 MNOP). Transfer of bacteria from tile was between 1.17 (14.62%) to 1.96 log % transfer (90.52%) (Table 3, Figure 2M). Greater transfer at 0 s was observed from stainless steel and wood (Figure 2NO) with transfer of 1.96 (91.16%) log % transfer and 1.93 log % transfer (85.97%) to watermelon, respectively (Table 3, Figure 2NO). Transfer from carpet ranged from -0.75 (0.18%) to 0.14 log % transfer (1.39%) (Table 3, Figure 2P).

II.4.e Inoculated surface to bread

E. aerogenes transfer from inoculated surfaces to bread is shown in Table 4 and Figure 1. When bread was dropped on TSB inoculated tile, stainless steel, wood or carpet, the highest transfer rate was observed at 30 s from wood (Figure 1C), although a significant

Table 3. Statistical analysis of contact time and inoculum matrix on Log % transfer of *Enterobacter aerogenes* from common household surfaces to watermelon.

Inoculated Surface	Contact time (s)	Inoculum Matrix	$\bar{x}$	σ	Median	Min	Max	Range	Below detection
Stainless Steel	0	TSB	1.96	0.01	1.96	1.93	1.98	0.05	0/20
		Buffer	1.96	0.01	1.96	1.93	1.99	0.06	0/20
	5	TSB	1.96	0.01	1.96	1.93	1.98	0.05	0/20
		Buffer	1.98	0.01	1.98	1.96	1.99	0.03	0/20
	30	TSB	1.96	0.01	1.96	1.95	1.98	0.03	0/20
		Buffer	1.98	0.01	1.98	1.96	1.99	0.03	0/20
	300	TSB	1.97	0.01	1.97	1.95	1.98	0.03	0/20
		Buffer	1.99	0.00	1.99	1.98	1.99	0.01	0/20
Tile	0	TSB	1.98	0.01	1.98	1.95	1.99	0.04	0/20
		Buffer	1.17	0.21	1.17	0.68	1.54	0.86	0/20
	5	TSB	1.99	0.00	1.99	1.98	1.99	0.02	0/20
		Buffer	1.52	0.09	1.53	1.33	1.66	0.33	0/20
	30	TSB	1.99	0.00	1.99	1.98	1.99	0.01	0/20
		Buffer	1.61	0.09	1.63	1.39	1.74	0.35	0/20
	300	TSB	1.98	0.00	1.98	1.98	1.99	0.01	0/20
		Buffer	1.96	0.02	1.97	1.92	1.98	0.06	0/20
Wood	0	TSB	1.96	0.04	1.98	1.84	1.99	0.15	0/20
		Buffer	1.93	0.04	1.95	1.82	1.98	0.16	0/20
	5	TSB	1.96	0.03	1.97	1.88	1.99	0.11	0/20
		Buffer	1.96	0.01	1.96	1.93	1.98	0.04	0/20
	30	TSB	1.96	0.01	1.96	1.95	1.98	0.03	0/20
		Buffer	1.97	0.01	1.97	1.95	1.98	0.03	0/20
	300	TSB	1.97	0.02	1.97	1.93	1.99	0.06	0/20
		Buffer	1.97	0.01	1.97	1.94	1.99	0.05	0/20
Carpet	0	TSB	1.91	0.03	1.91	1.82	1.98	0.17	0/20
		Buffer	-0.75	0.36	-0.88	-1.11	0.12	1.23	0/20
	5	TSB	1.93	0.02	1.93	1.89	1.96	0.07	0/20
		Buffer	-0.65	0.23	-0.69	-0.94	-0.09	0.85	0/20
	30	TSB	1.95	0.01	1.94	1.92	1.97	0.05	0/20
		Buffer	-0.69	0.17	-0.75	-0.87	-0.29	0.58	0/20
	300	TSB	1.94	0.02	1.94	1.89	1.97	0.08	0/20
		Buffer	0.14	0.41	0.19	-0.84	0.66	1.50	0/20

Table 4. Statistical analysis of contact time and inoculum matrix on Log % transfer of *Enterobacter aerogenes* from common household surfaces to bread.

Inoculated Surface	Contact time (s)	Inoculum Matrix	$\bar{x}$	σ	Median	Min	Max	Range	Below detection
Stainless Steel	0	TSB	-0.56	1.14	-0.69	-1.88	1.40	3.28	0/20
		Buffer	-1.24	0.30	-1.25	-1.60	-0.59	1.01	10/20
	5	TSB	1.92	0.07	1.95	1.72	1.99	0.27	0/20
		Buffer	1.16	0.49	1.17	-0.14	1.90	2.04	0/20
	30	TSB	1.97	0.04	1.98	1.85	1.99	0.15	0/20
		Buffer	1.67	0.28	1.74	0.75	1.95	1.20	0/20
	300	TSB	1.92	0.10	1.97	1.61	2.00	0.39	0/20
		Buffer	1.91	0.14	1.96	1.36	2.00	0.64	0/20
Tile	0	TSB	-0.95	0.43	-1.02	-1.57	-0.02	1.55	9/20
		Buffer	-0.68	0.83	-0.79	-2.19	0.84	3.03	16/20
	5	TSB	1.27	0.32	1.30	0.45	1.70	1.25	0/20
		Buffer	1.06	0.59	0.96	0.24	1.99	1.75	0/20
	30	TSB	1.96	0.04	1.98	1.87	2.00	0.12	0/20
		Buffer	1.49	0.35	1.51	0.86	1.92	1.07	0/20
	300	TSB	1.95	0.07	1.98	1.70	1.99	0.30	0/20
		Buffer	1.79	0.15	1.77	1.54	1.99	0.45	0/20
Wood	0	TSB	-0.64	1.16	-0.86	-2.03	0.92	2.95	8/20
		Buffer	-0.91	0.56	-1.05	-1.73	0.13	1.85	11/20
	5	TSB	1.89	0.12	1.94	1.58	1.98	0.41	0/20
		Buffer	0.37	0.81	0.39	-0.97	1.75	2.72	5/20
	30	TSB	1.97	0.02	1.98	1.91	2.00	0.08	0/20
		Buffer	1.21	0.55	1.26	0.03	1.97	1.94	0/20
	300	TSB	1.95	0.08	1.97	1.66	2.00	0.34	0/20
		Buffer	1.89	0.11	1.93	1.59	1.99	0.40	0/20
Carpet	0	TSB	-0.87	0.71	-0.91	-2.08	1.47	3.55	18/20
		Buffer	-1.68	0.15	-1.67	-1.91	-1.43	0.47	19/20
	5	TSB	-0.58	0.74	-0.62	-2.91	0.65	3.56	0/20
		Buffer	-0.89	0.60	-1.03	-1.81	0.82	2.64	17/20
	30	TSB	0.58	0.80	0.88	-1.97	1.61	3.58	0/20
		Buffer	-0.87	0.72	-0.83	-2.25	0.48	2.73	16/20
	300	TSB	0.55	0.79	0.49	-1.88	1.57	3.45	0/20
		Buffer	-0.79	0.60	-0.75	-2.02	0.24	2.26	1/20

Table 5. Statistical analysis of contact time and inoculum matrix on Log % transfer of *Enterobacter aerogenes* from common household surfaces to buttered bread.

Inoculated Surface	Contact time (s)	Inoculum Matrix	$\bar{x}$	σ	Median	Min	Max	Range	Below detection
Stainless Steel	0	TSB	-1.63	0.44	-1.76	-2.11	-0.72	1.39	13/20
		Buffer	-0.86	0.10	-0.89	-1.01	-0.63	0.38	18/20
	5	TSB	1.40	0.27	1.44	0.82	1.83	1.02	0/20
		Buffer	-0.67	0.21	-0.73	-0.97	-0.27	0.69	11/20
	30	TSB	1.36	0.26	1.45	0.81	1.67	0.86	0/20
		Buffer	-0.08	0.34	-0.09	-0.87	0.59	1.46	0/20
	300	TSB	1.91	0.06	1.93	1.79	1.99	0.19	0/20
		Buffer	1.42	0.19	1.43	0.97	1.81	0.83	0/20
Tile	0	TSB	-1.08	0.46	-1.12	-1.98	0.06	2.03	10/20
		Buffer	-0.86	0.11	-0.87	-1.02	-0.50	0.52	18/20
	5	TSB	1.35	0.42	1.46	0.54	1.92	1.37	0/20
		Buffer	-0.48	0.29	-0.48	-0.96	0.08	1.04	14/20
	30	TSB	1.58	0.24	1.66	1.11	1.85	0.75	0/20
		Buffer	1.28	0.27	1.34	0.50	1.62	1.12	18/20
	300	TSB	1.81	0.13	1.86	1.37	1.94	0.57	0/20
		Buffer	1.67	0.11	1.69	1.47	1.82	0.35	0/20
Wood	0	TSB	-1.18	0.33	-1.08	-1.93	-0.69	1.24	10/20
		Buffer	-0.29	0.42	-0.23	-0.95	0.37	1.32	9/20
	5	TSB	1.21	0.53	1.24	0.36	1.92	1.56	0/20
		Buffer	-0.25	0.52	-0.26	-0.92	0.68	1.60	6/20
	30	TSB	1.69	0.13	1.70	1.44	1.87	0.42	0/20
		Buffer	0.08	0.45	0.23	-0.83	0.58	1.41	1/20
	300	TSB	1.81	0.11	1.84	1.53	1.98	0.45	0/20
		Buffer	1.48	0.12	1.50	1.21	1.68	0.47	0/20
Carpet	0	TSB	-1.15	0.45	-0.99	-1.92	-0.44	1.49	10/20
		Buffer	-0.56	0.20	-0.57	-0.81	0.06	0.87	19/20
	5	TSB	-0.37	0.43	-0.47	-1.05	0.49	1.54	7/20
		Buffer	-0.61	0.16	-0.59	-0.84	-0.25	0.59	19/20
	30	TSB	-0.25	0.44	-0.13	-0.89	0.29	1.18	4/20
		Buffer	-0.23	0.25	-0.21	-0.67	0.43	1.10	12/20
	300	TSB	0.90	0.37	0.86	0.17	1.62	1.45	1/20
		Buffer	0.19	0.36	0.14	-0.29	1.00	1.29	0/20

Table 6. Statistical analysis of contact time and inoculum matrix on Log % transfer of *Enterobacter aerogenes* from common household surfaces to gummy candy.

Inoculated Surface	Contact time (s)	Inoculum Matrix	$\bar{x}$	σ	Median	Min	Max	Range	Below detection
Stainless Steel	0	TSB	-0.92	0.25	-0.93	-1.25	-0.10	1.15	17/20
		Buffer	-1.37	0.28	-1.39	-1.87	-0.84	1.04	19/20
	5	TSB	-0.74	0.28	-0.74	-1.38	-0.23	1.15	16/20
		Buffer	-1.38	0.41	-1.45	-2.10	-0.43	1.66	13/20
	30	TSB	-0.75	0.52	-0.92	-1.25	0.94	2.19	16/20
		Buffer	-1.36	0.38	-1.41	-1.99	-0.67	1.31	12/20
	300	TSB	1.80	0.11	1.84	1.54	1.93	0.39	9/20
		Buffer	-1.01	0.37	-1.00	-1.76	-0.30	1.46	7/20
Tile	0	TSB	-0.88	0.25	-0.91	-1.21	-0.40	0.81	18/20
		Buffer	-1.46	0.30	-1.52	-1.85	-0.80	1.06	19/20
	5	TSB	-0.50	0.48	-0.62	-1.24	0.47	1.71	18/20
		Buffer	-1.21	0.42	-1.35	-1.68	-0.27	1.41	17/20
	30	TSB	-0.45	0.45	-0.60	-0.96	0.84	1.80	16/20
		Buffer	-1.23	0.42	-1.30	-1.80	0.15	1.94	13/20
	300	TSB	0.28	0.75	0.33	-0.96	1.65	2.61	0/20
		Buffer	-0.89	0.48	-0.80	-1.77	-0.22	1.55	8/20
Wood	0	TSB	0.22	1.01	0.13	-1.03	1.68	2.70	8/20
		Buffer	-1.13	0.45	-1.30	-1.53	-0.10	1.43	19/20
	5	TSB	-1.04	0.24	-1.06	-1.49	-0.57	0.92	18/20
		Buffer	-1.16	0.29	-1.09	-1.67	-0.67	1.00	19/20
	30	TSB	-0.76	0.44	-0.82	-1.46	0.31	1.77	14/20
		Buffer	-1.18	0.24	-1.22	-1.52	-0.77	0.75	19/20
	300	TSB	0.34	0.45	0.38	-0.62	1.21	1.83	0/20
		Buffer	-1.20	0.21	-1.27	-1.48	-0.76	0.72	19/20
Carpet	0	TSB	-0.42	0.78	-0.66	-1.20	1.47	2.66	18/20
		Buffer	-1.27	0.17	-1.26	-1.58	-0.90	0.68	19/20
	5	TSB	-0.63	0.49	-0.82	-1.46	0.30	1.76	14/20
		Buffer	-1.34	0.17	-1.31	-1.78	-1.12	0.65	19/20
	30	TSB	-0.63	0.37	-0.71	-1.14	0.55	1.69	14/20
		Buffer	-1.36	0.20	-1.42	-1.68	-0.92	0.76	19/20
	300	TSB	-0.51	0.54	-0.69	-1.25	0.77	2.02	12/20
		Buffer	-1.22	0.19	-1.30	-1.42	-0.76	0.65	19/20

Figure 1. The effect of contact time on Log % transfer of *Enterobacter aerogenes* inoculated onto four household surfaces in a tryptic soy broth matrix to four foods.

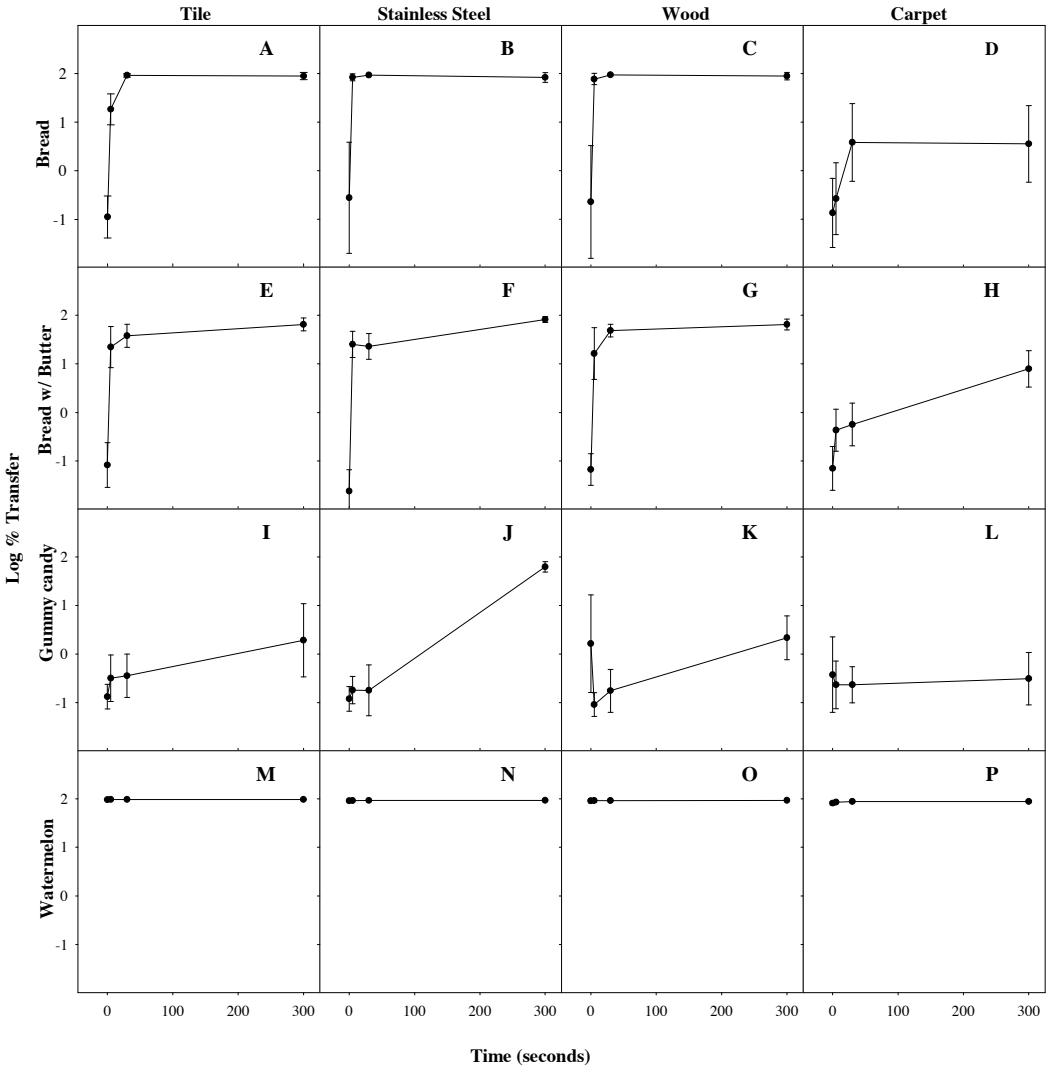
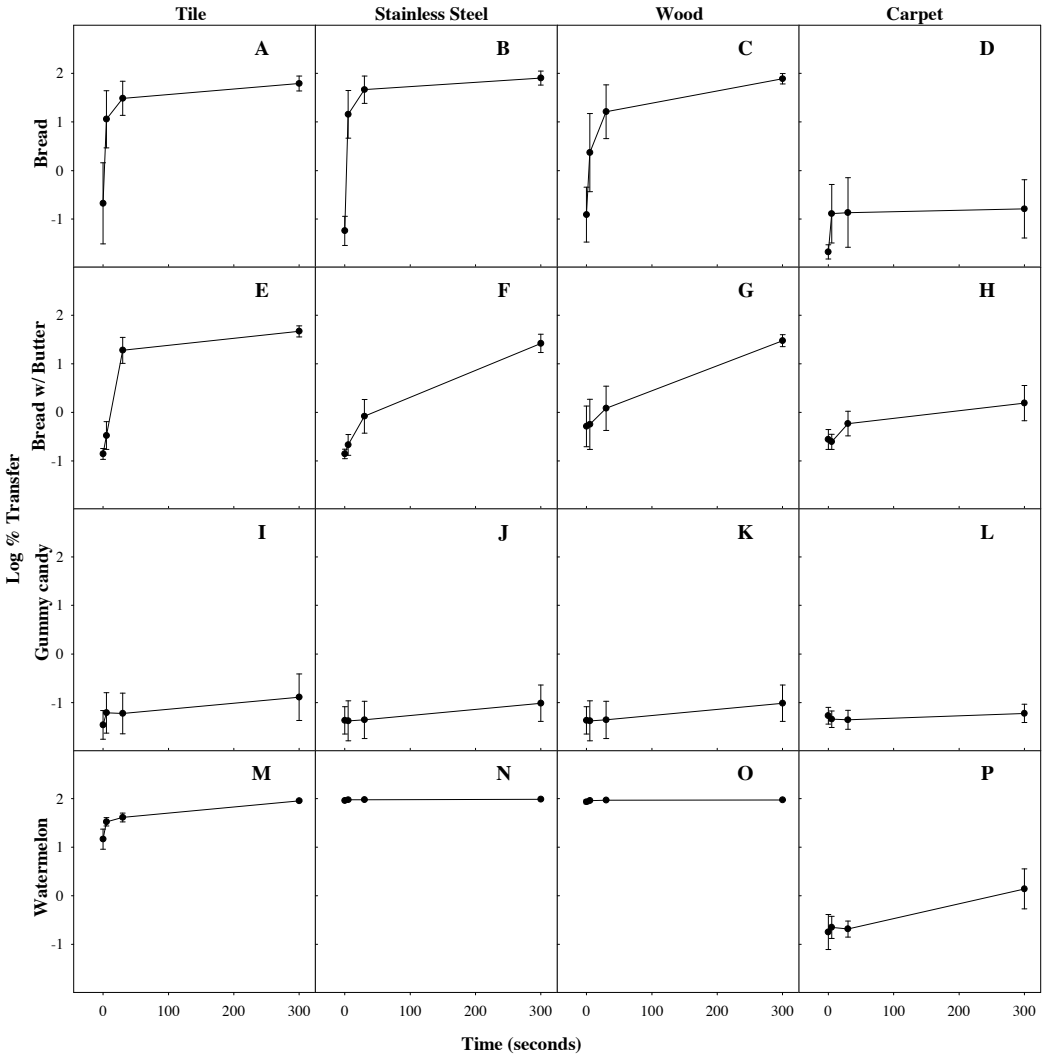


Figure 2. The effect of contact time on Log % transfer of *Enterobacter aerogenes* inoculated onto four household surfaces in a peptone buffer matrix to four foods.



difference between transfer at 30 and 300 s was not observed from wood (Table 4). Transfer of bacteria from stainless steel was between -0.56 (0.28%) and 1.97 log % transfer (93.11%) (Table 4, Figure 1B). For bread dropped on tile, the transfer ranged from -0.95 (0.11%) to 1.96 log % transfer (92.02%) (Table 4, Figure 1A), and transfer from wood ranged was -0.64 (0.23%) to 1.97 log % transfer (94.03%) (Table 4, Figure 1C). Transfer from carpet ranged from -0.87 (0.13%) to 0.58 log % transfer (3.82%), was less in comparison to the other three contact surfaces (Table 4, Figure 1D).

E. aerogenes transfer from Buffer inoculated surfaces to bread is shown in Table 4 and Figure 2. Bread dropped on the surfaces behaved similarly regardless of peptone buffer or TSB inoculum matrix. The transfer of bacteria from buffer inoculated surfaces was highest at 300 s for all surfaces (Figure 2). Transfer of bacteria from tile to bread was between -0.68 (0.21%) and 1.79 log % transfer (61.99%) (Table 4, Figure 2A). Stainless steel had the highest transfer of bacteria to bread after 300 s at 1.91 log % transfer (80.42 %) (Table 4, Figure 2B). Transfer of bacteria from wood over time was between -0.91 (0.12%) and 1.89 log % transfer (77.58%) (Table 4, Figure 2C) and transfer of bacteria from carpet was -1.68 (0.02%) and -0.79 log % transfer (0.16%) (Table 4, Figure 2D).

II.4.f Inoculated surface to bread with butter

E. aerogenes transfer data from inoculated surfaces to bread with butter is shown in Table 5. Bread with butter behaved similarly to bread when surfaces were inoculated with TSB (Figure 1, Table 5). Bacteria transfer from all surfaces to bread with butter at 0 s was low; on average, 10/20 replicates were below the detection limit (Table 5) where the detection limit was 2 log % transfer based on the protocols used in our experiments. When bread with butter was in contact with inoculated tile, transfer of bacteria increased from 0 to 300 s with mean log % transfer between -1.08 (0.08%) and 1.81 log % transfer

(64.92%) (Table 5, Figure 1E). The transfer of bacteria from stainless steel to bread with butter was between -1.63 (0.02%) and 1.91 log % transfer (82.20%) (Table 5, Figure 1F) and transfer from wood to bread with butter was between -1.18 (0.07%) and 1.81 log % transfer (65.19%) (Table 5, Figure 1G). Carpet transferred fewer bacteria in comparison to the other contact surfaces; yet transfer still increased over time from -1.15 (0.07%) to 0.9 log % transfer (7.92%) (Table 5, Figure 1H).

Transfer of *E. aerogenes* from Buffer inoculated surfaces to bread with butter is shown in Figure 2 and Table 5. There was an increase in bacterial transfer for all surfaces as contact time increased. Tile inoculated with cells contained in Buffer transferred more bacteria to bread with butter than any other surface (Figure 2). When bread with butter contacted tile, transfer of bacteria ranged from -0.86 (0.14%) to 1.67 log % transfer (46.60%) (Table 5, Figure 2E). Stainless steel and wood transferred a similar fraction of cells contained in Buffer to bread with butter (Table 5). Stainless steel transferred -0.86 (0.14%) and 1.42 log % transfer (26.22%) at 0 to 300 s (Table 5, Figure 2F) respectively, while wood transfer rates ranged from -0.29 (0.51%) to 1.48 log % transfer (29.88%) (Table 5, Figure 2G). Carpet again showed the lowest transfer rates ranging from -0.56 (0.28%) to 0.19 log % transfer (1.55%) (Table 5, Figure 2H).

II.4.g Inoculated surface to gummy candy

Transfer of *E. aerogenes* from surfaces inoculated in a TSB matrix to gummy candies is shown in Figure 1 and Table 6. Transfer rate increased with time from tile, ranging from -0.88 (0.13%) to 0.28 log % transfer (1.92%) (Table 6, Figure 1I). Transfer was lowest at 300 s from carpet to gummy candies with a -0.51 log % transfer (0.31%) (Table 6, Figure 1L). The transfer from stainless steel increased over time from 0 to 300 s, although, at 0, 5 and 30 s, on average 16/20 replicates were below the detection limit (Table 6, Figure

1J). The highest transfer observed for any surface to gummy candy occurred at 300 s from stainless steel to gummy with 1.80 log % transfer (62.62%) (Table 6, Figure 1J). When gummy candies were dropped on all surfaces containing the inoculum in buffer, the log % transfer was low, regardless of time (Table 6). On average, 19/20 replicates for gummy to all surfaces at 0 s were below the detection limit and an average of 8/20 were below the detection limit at 300 s (Table 6). The highest transfer was observed at 300 s from tile with bacterial transfer of -0.89 log % transfer (0.13%) (Table 6, Figure 2I).

II.5 Discussion

Our study shows that bacterial transfer is dependent on the surface, food type, contact time and inoculum matrix. Studies involving transfer of similar surfaces to foods have come to varying conclusions (*29*, *51*). These differences may be due to the range of experimental procedures among published studies. Differences include the contact time between surfaces (*29*, *51*, *75*), organism used (*29*, *51*, *58*, *75*) and food and contact surfaces used (*29*, *51*, *58*, *75*) which can result in differing outcomes. Our research also shows that the nature of the matrix containing the cells inoculated onto the surface can play an important role, even when all other experimental variables are the same, an observation we have seldom seen reported in literature (*30*). Studies reporting on bacterial adhesion to surfaces use a variety of drying times, in comparison to the 5 h drying time used in this study (*29*, *32*, *51*, *88*). Additionally, there is a difference in data analysis regarding transfer rates. Some studies determined transfer rate by recipient surface/source surface (*24*), whereas in our study, transfer rate was analyzed by recipient surface/(source surface + recipient surface) (*29*, *51*, *75*), which can lead to slight differences when the number of bacteria transferred to the recipient surface is high. More

importantly, some studies use very small numbers of replicates and/or fail to statistically transform the percent transfer rates, and may come to erroneous conclusions (*24*, *92*, *109*). Although not always reported in studies, standard deviation is a good indication of the degree of variability (*24*). In our study, the standard deviation varies considerably based on the food. With more replicates, the standard deviation of the population will be more precise.

Although pressure was not a variable in our study, it may play a role in facilitating bacterial transfer. Kusumaningrum et al. found that more transfer occurred when light pressure was applied (20 g/cm^2), although differences were slight (~0.3-log percent transfer difference) (*58*). Mbithi et al. used pressures of 200 and 1,000 g/cm^2, with and without friction and found that differences in transfer rates were also small (a ~0.5-log percent transfer difference when pressure is applied) (*70*). Research by D'Souza et al 2006 showed that pressure changes from ~1 to 100 g/cm^2 had no effect on virus transfer (*33*). Later research from the same laboratory showed more transfer at higher pressures (~100 g/cm^2) compared with lower pressures (~10 g/cm^2), especially where the inoculum was drier (*34*).

Our data clearly showed that contact time does influence bacterial transfer, with more bacteria transferred at longer times. Peer reviewed research by Dawson et al. reported that longer food contact times (5, 30 or 60 s) did result in greater transfer but only at longer drying times (≥ 8 h) (*29*) which is roughly equivalent to our drying time of 5 h. Non-peer reviewed research from the University of Illinois on bacterial transfer from tile inoculated with generic *E. coli* to cookies and gummy bears and found that bacterial transfer was observed in less than 5 seconds (*4*) (consistent with our 0 sec observations)

although other contact times were not studied. The popular television show MythBusters (*5*) aired an episode on the "five second rule" and found no conclusive difference when pastrami and crackers were exposed to contaminated tile with contact times of 2 and 6 seconds. It is unclear from viewing the episode what was used to contaminate the tile surface, although the inoculated tile was left for 5 days before beginning the experiment. Mythbusters also used less than 10 replicates per scenario. A press release by Aston University, in the United Kingdom, showed that time significantly affected transfer depending on the contaminated surface and food (*42*). The Aston University study observed the transfer of *E. coli* and *S. aureus* from carpet, wood and tile to toast, pasta, biscuit and a sticky sweet with 3 and 30 s contact time. Moist foods that contacted contaminated wood and tile showed higher transfer rates, and longer times increased transfer for these foods and surfaces. The Aston University study shows that transfer from carpet was not affected by the food composition or the contact time (*42*).

Our data show that the rate of bacterial transfer was greatest for tile, stainless steel and wood surfaces at 300 s. The food with the highest transfer rate was watermelon, regardless of contact time, which may be due to several factors. When watermelon is cut, it is very moist, and moisture is known to facilitate transfer (*80*), regardless of whether the contact surface is dry or wet. Watermelon may also present a flatter, more uniform surface at the microscopic level compared to bread or gummy candies. Jensen et al. also found that transfer from stainless steel and tile to watermelon had the highest transfer in comparison to the other produce types used in that study (*51*). Kusumaningrum et al. measured the transfer rates to cut cucumber from stainless steel, and observed that almost all bacteria (~100%) transferred to the cucumber regardless of pressure (*58*). Cut

cucumbers also have a moist, uniform surface, which may facilitate bacterial transfer. We observed lower transfer rates (~0.2%) when transfer was from carpet to food. Carpet may promote less bacterial transfer because of bacterial attachment or infiltration into absorbent carpet fibers. Dawson et al. also found that transfer from carpet to bologna was very low (<0.5%) in comparison to the transfer from wood and tile to bologna (5-68%) (*29*).

The starting concentration of all surfaces in our experiments were ~7 log CFU/surface. Although this was not a variable explicitly considered, the starting concentration may have an affect on how much bacterial transfer occurs to the recipient surface. Montville and Schaffner reported on the influence of inoculum size on bacterial cross-contamination between surfaces. Their results showed that the effect of inoculum size on transfer rate was statistically significant ($P < 0.0001$) for all transfer rate data, and that greater inoculum size resulted in lower transfer rates (*74*).

Transfer of bacteria from surfaces to food appear to be most affected by the moisture of the food as show by transfer of *E. aerogenes* from tile, stainless steel, wood and carpet to watermelon. Longer food contact times usually resulted in transfer of more bacteria from each surface to food. Carpet has very low transfer rates, compared with tile and stainless steel, whereas transfer from wood was more variable. The topography of the surface and food seems to play an important role in bacterial transfer. The risk of illness resulting from deciding to consume food that has fallen on the floor will depend on factors including prevalence, concentration and type of organism, the nature of the food (especially moisture), the nature of the surface topology as well as the length of time the food is in contact with the surface. Although this research shows that the 5-second rule is

"real" in the sense that longer contact time result in more transfer, it also shows that other factors including the nature of the food and the surface are equally important.

References

1. Abrishami, S. H., B. D. Tall, T. J. Bruursema, P. S. Epstein, and D. B. Shah. 1994. Bacterial adherence and viability on cutting board surfaces. *J. Food Safety.* 14:153-172.
2. Ak, N. O., D. O. Cliver, and C. W. Kaspar. 1994. Cutting boards of plastic and wood contaminated experimentally with bacteria. *J. Food Prot.* 57:16-22.
3. Anderson, R. L., D. C. Mackel, B. S. Stoler, and G. F. Mallison. 1982. Carpeting in hospitals: an epidemiological evaluation. *J. Clin. Microbiol.* 15:408-415.
4. Anonymous. 2003. If You Drop It, Should You Eat It? Scientists Weigh In on the 5-Second Rule. http://news.aces.illinois.edu/news/if-you-drop-it-should-you-eat-it-scientists-weigh-5-second-rule. Accessed 15 February 2016.
5. Anonymous. 2005. 5 Second Rule With Food On Floor. http://www.discovery.com/tv-shows/mythbusters/mythbusters-database/5-second-rule-with-food/. Accessed 4 February 2016.
6. Ayliffe, G. A., B. J. Collins, E. J. Lowbury, J. R. Babb, and H. A. Lilly. 1967. Ward floors and other surfaces as reservoirs of hospital infection. *J. Hyg. (Lond).* 65:515-536.
7. Baev, D., R. England, and H. K. Kuramitsu. 1999. Stress-induced membrane association of the *Streptococcus mutans* GTP-binding protein, an essential G protein, and investigation of its physiological role by utilizing an antisense RNA strategy. *Infect. Immun.* 67:4510-4516.
8. Behrsing, J., J. Jaeger, F. Horlock, N. Kita, P. Franz, and R. Premier. 2003. Survival of *Listeria innocua*, *Salmonella salford* and *Escherichia coli* on the surface of fruit with inedible skins. *Postharvest Biol.Technol.* 29:249-256.
9. Berto, A. M. 2007. Ceramic tiles: Above and beyond traditional applications. *J. Eur. Ceram. Soc.* 27:1607-1613.
10. Bloomfield, S. F., and E. Scott. 1997. Cross-contamination and infection in the domestic environment and the role of chemical disinfectants. *J. Appl. Microbiol.* 83:1-9.
11. Blostein, J. 1993. An outbreak of *Salmonella javiana* associated with consumption of watermelon. *J. Environ. Health.* 56:29-31.
12. Bowen, A., A. Fry, G. Richards, and L. Beuchat. 2006. Infections associated with cantaloupe consumption: a public health concern. *Epidemiol. Infect.* 134:675-685.
13. Bryan, F. L. 1988. Risks of practices, procedures and processes that lead to outbreaks of foodborne diseases. *J. Food Prot.* 51:663-673.
14. Campbell, G. M., and E. Mougeot. 1999. Creation and characterisation of aerated food products. *Trends Food Sci. Technol.* 10:283-296.
15. Centers for Disease Control and Prevention (CDC). 1970. Staphylococcal food poisoning traced to butter-Alabama. *Morb. Mortal. Wkly. Rep.* 19:271.
16. Centers for Disease Control and Prevention (CDC). 1979. *Salmonella* Oranienburg gastroenteritis associated with consumption of precut watermelons: Illinois. *Morb. Mortal. Wkly. Rep.* 28:522-523.
17. Centers for Disease Control and Prevention (CDC). 1991. Epidemiologic Notes and Reports Cholera - New Jersey and Florida. *Morb. Mortal. Wkly. Rep.* 40:287-289.
18. Centers for Disease Control and Prevention (CDC). 2012. Multistate Outbreak of *Salmonella* Typhimurium and *Salmonella* Newport Infections Linked to Cantaloupe (Final Update). http://www.cdc.gov/salmonella/typhimurium-cantaloupe-08-12/. Accessed 16 February 2016.

19. Centers for Disease Control and Prevention (CDC). 2013. Surveillance for foodborne disease outbreaks - United States, 2009-2010. *Morb. Mortal. Wkly. Rep.* 62:41-47.
20. Centers for Disease Control and Prevention (CDC). 2013. Surveillance for Foodborne Disease Outbreaks United States, 2011: Annual Report. http://www.cdc.gov/foodsafety/pdfs/foodborne-disease-outbreaks-annual-report-2011-508c.pdf.
21. Centers for Disease Control and Prevention (CDC). 2014. Surveillance for Foodborne Disease Outbreaks United States, 2012: Annual Report. http://www.cdc.gov/foodsafety/pdfs/foodborne-disease-outbreaks-annual-report-2012-508c.pdf.
22. Centers for Disease Control and Prevention (CDC). 2015. Surveillance for Foodborne Disease Outbreaks United States, 2013: Annual Report. http://www.cdc.gov/foodsafety/pdfs/foodborne-disease-outbreaks-annual-report-2013-508c.pdf.
23. Cheesbrough, J. S., L. Barkess-Jones, and D. W. Brown. 1997. Possible prolonged environmental survival of small round structured viruses. *J. Hosp. Infect.* 35:325-326.
24. Chen, Y., K. M. Jackson, F. P. Chea, and D. W. Schaffner. 2001. Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks. *J. Food Prot.* 64:72-80.
25. Cheng, G., Z. Zhang, S. Chen, J. D. Bryers, and S. Jiang. 2007. Inhibition of bacterial adhesion and biofilm formation on zwitterionic surfaces. *Biomaterials.* 28:4192-4199.
26. Corpe, W. A. 1980. Microbial surface components involved in adsorption of microorganisms onto surfaces. *Adsorption of microorganisms to surfaces*. John Wiley & Sons, Inc., New York. 105-144.
27. Czuchajowska, Z., Y. Pomeranz, and H. C. Jeffers. 1989. Water activity and moisture content of dough and bread. *Cereal Chem. (USA).*
28. Danyluk, M. D., L. M. Friedrich, and D. W. Schaffner. 2014. Modeling the growth of *Listeria monocytogenes* on cut cantaloupe, honeydew and watermelon. *Food Microbiol.* 38:52-55.
29. Dawson, P., I. Han, M. Cox, C. Black, and L. Simmons. 2007. Residence time and food contact time effects on transfer of *Salmonella* Typhimurium from tile, wood and carpet: testing the five-second rule. *J. Appl. Microbiol.* 102:945-953.
30. Dickson, J. S., G. R. Siragusa, and J. E. Wray. 1992. Predicting the growth of *Salmonella typhimurium* on beef by using the temperature function integration technique. *Appl. Environ. Microbiol.* 58:3482-3487.
31. Donlan, R. M. 2001. Biofilm formation: a clinically relevant microbiological process. *Clin. Infect. Dis.* 33:1387-1392.
32. Donlan, R. M. 2002. Biofilms: microbial life on surfaces. *Emerg. Infect. Dis.* 8:881-890.
33. D'Souza, D. H., A. Sair, K. Williams, E. Papafragkou, J. Jean, C. Moore, and L. Jaykus. 2006. Persistence of caliciviruses on environmental surfaces and their transfer to food. *Int. J. Food Microbiol.* 108:84-91.
34. Escudero, B. I., H. Rawsthorne, C. Gensel, and L. A. Jaykus. 2012. Persistence and Transferability of Noroviruses on and between Common Surfaces and Foods. *J. Food Prot.* 75:927-935.

35. Fan, X. 2009. Microbial safety of fresh produce. IFT Press; Ames, Iowa: Wiley-Blackwell, Chicago.
36. Farmiloe, F. J., S. J. Cornford, J. B. M. Coppock, and M. Ingram. 1954. The survival of *Bacillus subtilis* spores in the baking of bread. *J. Sci. Food Agric.* 5:292-304.
37. Fattom, A., and M. Shilo. 1984. Hydrophobicity as an adhesion mechanism of benthic cyanobacteria. *Appl. Environ. Microbiol.* 47:135-143.
38. Fein, S. B., C. T. J. Lin, and A. S. Levy. 1995. Foodborne illness: perceptions, experience, and preventive behaviors in the United States. *J. Food Prot.* 58:1405-1411.
39. Fernandez-Salguero, J., R. Gómez, and M. A. Carmona. 1993. Water activity in selected high-moisture foods. *J. Food Comp. Anal.* 6:364-369.
40. Fletcher, M., and G. I. Loeb. 1979. Influence of substratum characteristics on the attachment of a marine pseudomonad to solid surfaces. *Appl. Environ. Microbiol.* 37:67-72.
41. Food and Agricultural Organization of the United Nations (FAO). 2011. Microbial hazards and melons: Codex Committee on Food Hygiene Working Group on the development of an annex on methods for the Code of Hygienic Practice for fresh fruits and vegetables (CAC/RCP 53-2003). ftp://ftp.fao.org/ag/agn/jemra/Microbiological_hazards_and_melons_Nov08.pdf..
42. Garbett, J. 2014. Researchers prove the five-second rule is real. http://www.aston.ac.uk/about/news/releases/2014/march/five-second-food-rule-does-exist/. Accessed 26 January 2016.
43. Garrett, T. R., M. Bhakoo, and Z. Zhang. 2008. Bacterial adhesion and biofilms on surfaces. *Prog. Nat. Sci.* 18:1049-1056.
44. Gilbert, R. J., and H. M. Watson. 1971. Some laboratory experiments on various meat preparation surfaces with regard to surface contamination and cleaning. *Int. J. Food Sci Tech.* 6:163-170.
45. Goldberg, J. 2002. Biofilms and antibiotic resistance: a genetic linkage. *Trends Microbiol.* 10:264.
46. Gould, L. H., K. A. Walsh, A. R. Vieira, K. Herman, I. T. Williams, A. J. Hall, and D. Cole. 2013. Surveillance for foodborne disease outbreaks - United States, 1998-2008. *Morbid. Mortal. Wkly. Rep. Surveill. Summ.* 62:1-34.
47. Greco, J. T. 1965. Carpeting vs. Resilient Flooring: A Comparative Study In A Metropolitan Hospital. *Hospitals.* 39:55-58.
48. Haiko, J., and B. Westerlund-Wikström. 2013. The role of the bacterial flagellum in adhesion and virulence. *Biology.* 2:1242-1267.
49. Holah, J. T., and R. H. Thorpe. 1990. Cleanability in relation to bacterial retention on unused and abraded domestic sink materials. *J. Appl. Bacteriol.* 69:599-608.
50. Janjaroen, D., F. Q. Ling, F. Ling, G. Monroy, N. Derlon, E. Morgenroth, E. Mogenroth, S. A. Boppart, W. T. Liu, and T. H. Nguyen. 2013. Roles of ionic strength and biofilm roughness on adhesion kinetics of *Escherichia coli* onto groundwater biofilm grown on PVC surfaces. *Water Res.* 47:2531-2542.
51. Jensen, D. A., L. M. Friedrich, L. J. Harris, M. D. Danyluk, and D. W. Schaffner. 2013. Quantifying transfer rates of *Salmonella* and *Escherichia coli* O157:H7 between fresh-cut produce and common kitchen surfaces. *J. Food Prot.* 76:1530-1538.

52. Karunakaran, E., J. Mukherjee, B. Ramalingam, and C. A. Biggs. 2011. "Biofilmology": a multidisciplinary review of the study of microbial biofilms. *Appl. Microbiol. Biotechnol.* 90:1869-1881.
53. Khambaty, F. M., R. W. Bennett, and D. B. Shah. 1994. Application of pulsed-fields gel electrophoresis to the epidemiological characterization of *Staphylococcus intermedius* implicated in a food-related outbreak. *Epidemiol. Infect.* 113:75-81.
54. Kimura, A. C., M. S. Palumbo, H. Meyers, S. Abbott, R. Rodriguez, and S. B. Werner. 2005. A multi-state outbreak of *Salmonella* serotype Thompson infection from commercially distributed bread contaminated by an ill food handler. *Epidemiol. Infect.* 133:823-828.
55. Klontz, K. C., B. Timbo, S. Fein, and A. Levy. 1995. Prevalence of selected food consumption and preparation behaviors associated with increased risks of food-borne disease. *J. Food Prot.* 58:927-930.
56. Koczan, J. M., B. R. Lenneman, M. J. McGrath, and G. W. Sundin. 2011. Cell surface attachment structures contribute to biofilm formation and xylem colonization by *Erwinia amylovora*. *Appl. Environ. Microbiol.* 77:7031-7039.
57. Kuhn, P. J. 1983. Doorknobs: a source of nosocomial infection. *Diagnost. Med.* 62-63.
58. Kusumaningrum, H. D., G. Riboldi, W. C. Hazeleger, and R. R. Beumer. 2003. Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *Int. J. Food Microbiol.* 85:227-236.
59. Kusumaningrum, H. D., E. D. vanAsselt, R. R. Beumer, and M. H. Zwietering. 2004. A quantitative analysis of cross-contamination of *Salmonella* and *Campylobacter* spp. via domestic kitchen surfaces. *J. Food Prot.* 67:1892-1903.
60. Lankford, M. G., S. Collins, L. Youngberg, D. M. Rooney, J. R. Warren, and G. A. Noskin. 2006. Assessment of materials commonly utilized in health care: implications for bacterial survival and transmission. *Am. J. Infect. Control.* 34:258-263.
61. Lenovich, L., and P. Konkel. 1992. Confectionery Products. Compendium Methods for the Microbiological Examination of Foods
62. Litsky, B. Y., and W. Litsky. 1968. Investigations on decontamination of hospital surfaces by the use of disinfectant-detergents. *Am. J. Public Health Nations Health.* 58:534-543.
63. Legan, D. 2000. Cereals and Cereal Products. In *The Microbiological Safety and Quality of Food.* 1:759-783.
64. Lund, B. M., C. W. Donnelly, and A. Rampling. 2000. Heat resistance of *Mycobacterium paratuberculosis. Lett. Appl. Microbiol.* 31:184-185.
65. Lynch, M. F., R. V. Tauxe, and C. W. Hedberg. 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiol. Infect.* 137:307-315.
66. Lyytikäinen, O., T. Autio, R. Maijala, P. Ruutu, T. Honkanen-Buzalski, M. Miettinen, M. Hatakka, J. Mikkola, V. J. Anttila, T. Johansson, L. Rantala, T. Aalto, H. Korkeala, and A. Siitonen. 2000. An outbreak of *Listeria monocytogenes* serotype 3a infections from butter in Finland. *J. Infect. Dis.* 181:1838-1841.
67. Malik, Y. S., and S. M. Goyal. 2006. Virucidal efficacy of sodium bicarbonate on a food contact surface against feline calicivirus, a norovirus surrogate. *Int. J. Food Microbiol.* 109:160-163.

68. Marples, R. R., and A. G. Towers. 1979. A laboratory model for the investigation of contact transfer of microorganisms. *J. Hyg.* 82:237-248.
69. Marques, S. C., J. D. G. O. S. Rezende, L. A. F. de Alves, B. C. Silva, E. Alves, L. R. de Abreu, and R. H. Piccoli. 2007. Formation of biofilms by *Staphylococcus aureus* on stainless steel and glass surfaces and its resistance to some selected chemical sanitizers. *Braz. J. Microbiol.* 38:538-543.
70. Mbithi, J. N., V. S. Springthorpe, J. R. Boulet, and S. A. Sattar. 1992. Survival of hepatitis A virus on human hands and its transfer on contact with animate and inanimate surfaces. *J. Clin. Microbiol.* 30:757-763.
71. Merry, A. F., T. E. Miller, G. Findon, C. S. Webster, and S. P. Neff. 2001. Touch contamination levels during anaesthetic procedures and their relationship to hand hygiene procedures: a clinical audit. *Br. J. Anaesth.* 87:291-294.
72. Midelet, G., and B. Carpentier. 2002. Transfer of microorganisms, including *Listeria monocytogenes*, from various materials to beef. *Appl. Environ. Microbiol.* 68:4015-4024.
73. Mondal, A., and A. K. Datta. 2008. Bread baking-a review. *J. Food Eng.* 86:465-474.
74. Montville, R., and D. W. Schaffner. 2003. Inoculum size influences bacterial cross contamination between surfaces. *Appl. Environ. Microbiol.* 69:7188-7193.
75. Moore, C. M., B. W. Sheldon, and L. A. Jaykus. 2003. Transfer of *Salmonella* and *Campylobacter* from stainless steel to romaine lettuce. *J. Food Prot.* 66:2231-2236.
76. Moran, L. 2016. Science Explains Why The 5-Second Rule Is Actually True. http://www.huffingtonpost.com/entry/5-second-rule-science-explainer_us_56b07205e4b057d7d7c809a8. Accessed 4 February 2016.
77. Mylius, S. D., M. J. Nauta, and A. H. Havelaar. 2007. Cross-Contamination During Food Preparation: A Mechanistic Model Applied to Chicken-Borne *Campylobacter. Risk Analysis.* 27:803-813.
78. Ou, Y. -C., C. -W. Hsu, L. -J. Yang, H. -C. Han, Y. -W. Liu, and C. -Y. Chen. 2008. Attachment of tumor cells to the micropatterns of glutaraldehyde (GA)-crosslinked gelatin. *Sensors and Materials.* 20:435-446.
79. Peng, X. X., W. Luo, J. Y. Zhang, S. Y. Wang, and S. C. Lin. 2002. Rapid detection of *Shigella* species in environmental sewage by an immunocapture PCR with universal primers. *Appl. Environ. Microbiol.* 68:2580-2583.
80. Pérez-Rodríguez, F., A. Valero, E. Carrasco, R. M. García, and G. Zurera. 2008. Understanding and modelling bacterial transfer to foods: a review. *Trends Food Sci. Technol.* 19:131-144.
81. Pringle, J. H., and M. Fletcher. 1986. Influence of substratum hydration and adsorbed macromolecules on bacterial attachment to surfaces. *Appl. Environ. Microbiol.* 51:1321-1325.
82. Resnick, A. 2012. Life at the Nanoscale: Atomic Force Microscopy of Live Cells, edited by Yves Dufrêne: Scope: guide, handbook. Level: postgraduate, advanced undergraduate, early career researcher, researcher, specialist, scientist. *Contemporary Physics.* 53:377-377.
83. Rice, D. H., D. D. Hancock, M. H. Szymanski, B. C. Scheenstra, K. M. Cady, T. E. Besser, and P. A. Chudek. 2003. Household contamination with *Salmonella enterica. Emerg. Infect. Dis.* 9:120-122.

84. Richards, G. M., and L. R. Beuchat. 2005. Metabiotic associations of molds and *Salmonella* Poona on intact and wounded cantaloupe rind. *Int. J. Food Microbiol.* 97:327-339.
85. Richardson, T. 1987. ERH of confectionery food products. Manufacturing Conference. 67:65-70.
86. Robine, E., L. Boulangé-Petermann, and D. Derangère. 2002. Assessing bactericidal properties of materials: the case of metallic surfaces in contact with air. *J. Microbiol. Methods.* 49:225-234.
87. Rylander, R., K. E. Myrbäck, B. Verner-Carlson, and M. Ohrström. 1974. Bacteriological investigation of wall-to-wall carpeting. *Am. J. Public Health.* 64:163-168.
88. Ryu, J. H., and L. R. Beuchat. 2005. Biofilm formation by *Escherichia coli* O157:H7 on stainless steel: Effect of exopolysaccharide and curli production on its resistance to chlorine. *Appl. Environ. Microbiol.* 71:247-254.
89. Sattar, S. A., S. Springthorpe, S. Mani, M. Gallant, R. C. Nair, E. Scott, and J. Kain. 2001. Transfer of bacteria from fabrics to hands and other fabrics: development and application of a quantitative method using *Staphylococcus aureus* as a model. *J. Appl. Microbiol.* 90:962-970.
90. Scallan, E., P. M. Griffin, F. J. Angulo, R. V. Tauxe, and R. M. Hoekstra. 2011. Foodborne illness acquired in the United States - unspecified agents. *Emerg. Infect. Dis.* 17:16-22.
91. Scanlon, M. G., and M. C. Zghal. 2001. Bread properties and crumb structure. *Food Res. Int.* 34:841-864.
92. Schaffner, D. W. 2003. Challenges in cross contamination modelling in home and food service settings. *Food Aust.* 55:583-586.
93. Shaffer, J. G., and I. Key. 1966. The microbiological effects of carpeting on the hospital environment. *Hospitals.* 40:126-139.
94. Stevens, R. A., and J. T. Holah. 1993. The effect of wiping and spray-wash temperature on bacterial retention on abraded domestic sink surfaces. *J. Appl. Bacteriol.* 75:91-94.
95. Takeuchi, K., C. M. Matute, A. N. Hassan, and J. F. Frank. 2000. Comparison of the attachment of *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella typhimurium*, and *Pseudomonas fluorescens* to lettuce leaves. *J. Food Prot.* 63:1433-1437.
96. Tauxe, R., H. Kruse, C. Hedberg, M. Potter, J. Madden, and K. Wachsmuth. 1997. Microbial hazards and emerging issues associated with produce: a preliminary report to the National Advisory Committee on Microbiologic Criteria for Foods. *J. Food Prot.* 60:1400-1408.
97. Thompson, S. 2010. Microbiological spoilage of high-sugar products. In Compendium of the microbiological spoilage of foods and beverages, edited by W. H. Sperber, and M. P. Doyle. Springer, NY.
98. Todd, E. C., J. D. Greig, C. A. Bartleson, and B. S. Michaels. 2007. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 2. Description of outbreaks by size, severity, and settings. *J. Food Prot.* 70:1975-1993.
99. Torquato, S. 1998. Morphology and effective properties of disordered heterogeneous media. *Int. J. Solids Struct.* 35:2385-2406.

100. Tschape, H., R. Prager, W. Streckel, A. Fruth, E. Tietze, and G. Böhme. 1995. Verotoxinogenic *Citrobacter freundii* associated with severe gastroenteritis and cases of haemolytic uraemic syndrome in a nursery school: green butter as the infection source. *Epidemiol. Infect.* 114:441-450.
101. U.S. Department of Health, Education and Welfare. 1977. Presumed staphylococcal food poisoning associated with whipped butter. *Morb. Mortal. Wkly. Rep.* 26:268.
102. Ukuku, D. O., and W. F. Fett. 2002. Effectiveness of chlorine and nisin-EDTA treatments of whole melons and fresh-cut pieces for reducing native microflora and extending shelf-life. *J. Food Safety.* 22:231-253.
103. United States Department of Agriculture (USDA). 2013. Cutting Boards and Food Safety. http://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/safe-food-handling/cutting-boards-and-food-safety. Accessed 26 January 2016.
104. United States Food and Drug Administration (U.S. FDA). 1992. Blended margarine and butter products: Product is contaminated with *Staphylococcus* enterotoxin.
105. United States Food and Drug Administration (U.S. FDA). 2009. Guidance for Industry Guide to Minimize Microbial Food Safety Hazards of Melons. http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm174171.htm. Accessed 6 January 2016.
106. Vanlerberghe, B., P. Van Oostveldt, O. Thas, and P. Van der Meeren. 2008. Determination of water droplet size distribution in butter: pulsed field gradient NMR in comparison with confocal scanning laser microscopy. *Int. Dairy J.* 18:12-22.
107. van Loosdrecht, M. C., J. Lyklema, W. Norde, G. Schraa, and A. J. Zehnder. 1987. Electrophoretic mobility and hydrophobicity as a measured to predict the initial steps of bacterial adhesion. *Appl. Environ. Microbiol.* 53:1898-1901.
108. Vinnere Pettersson, O., and S. -L. L. Leong. 2011. Fungal Xerophiles (Osmophiles). *eLS.*
109. Wachtel, M. R., and A. O. Charkowski. 2002. Cross-contamination of lettuce with *Escherichia coli* O157:H7. *J. Food Prot.* 65:465-470.
110. Walter, W. G., A. H. Stober, and E. P. Smith. 1969. Studies of airborne bacteria in a partially carpeted hospital. *Health Lab. Sci.* 6:140-147.
111. Warburton, D. W., J. Farber, A. Armstrong, R. Caldeira, T. Hunt, S. Messier, R. Plante, N. P. Tiwari, and J. Vinet. 1991. A comparative study of the 'FDA' and 'USDA' methods for the detection of *Listeria monocytogenes* in foods. *Int. J. Food Microbiol.* 13:105-118.
112. Warren, W. E., and A. M. Kraynik. 1997. Linear elastic behavior of a low-density Kelvin foam with open cells. *J. Appl. Mech.* 64:787-794.
113. Welker, C., N. Faiola, S. Davis, I. Maffatore, and C. A. Batt. 1997. Bacterial retention and cleanability of plastic and wood cutting boards with commercial food service maintenance practices. *J. Food Prot.* 60:407-413.
114. Wendt, C., B. Dietze, E. Dietz, and H. Rüden. 1997. Survival of *Acinetobacter baumannii* on dry surfaces. *J. Clin. Microbiol.* 35:1394-1397.
115. Wilks, S. A., H. Michels, and C. W. Keevil. 2005. The survival of *Escherichia coli* O157 on a range of metal surfaces. *Int. J. Food Microbiol.* 105:445-454.

116. Wilks, S. A., H. T. Michels, and C. W. Keevil. 2006. Survival of *Listeria monocytogenes* Scott A on metal surfaces: Implications for cross-contamination. *Int. J. Food Microbiol.* 111:93-98.
117. Yu, C., V. J. Davidson, and S. X. Yang. 2006. A neural network approach to predict survival/death and growth/no-growth interfaces for *Escherichia coli* O157:H7. *Food Microbiol.* 23:552-560.
118. Zhao, T., M. P. Doyle, and D. E. Berg. 2000. Fate of *Campylobacter jejuni* in butter. *J. Food Prot.* 63:120-122.
119. Zhao, P., T. Zhao, M. P. Doyle, J. R. Rubino, and J. Meng. 1998. Development of a model for evaluation of microbial cross-contamination in the kitchen. *J. Food Prot.* 61:960-963.